Reviewer's report

Title: Antinociceptive tolerance effects of NSAIDs microinjected into dorsal hippocampus

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Reviewer: Abbas Haghparast

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The present manuscript entitled “Antinociceptive tolerance effects of NSAIDs microinjected into dorsal hippocampus” by Gurtskaia et al reports on the analgesia produced by local infusions of three different NSAIDs into the dorsal hippocampus of rats, and gradual decreases in the analgesia by repeated consecutive application of the drugs, representing tolerance phenomenon. They also showed that intra-hippocampal injection of naloxone reversed the effects produced by NSAIDs, particularly, on the first day of experiments. What’s more, pretreatment with naloxone abolished development of NSAID-induced antinociception.

The manuscript is well-written, concise, and easy-to-follow. The introduction is quite informative, and highlights the significance of the study. The results are interesting and extend existing knowledge on the underlying mechanisms of pain perception and modifications. Generally, I have a positive view on this work. However, there are some concerns mostly regarding the way the data were analyzed and presented, as follows:

1- Minor: the cannulas implanted into the dorsal hippocampi reported to be 12 mm long and were aimed at 2 mm above the target region. For placing a cannula 0.8 mm beneath the skull surface, a cannula 12 mm in length seems to be too long.

2- Minor: a Hamilton microsyringe of 50 µl was used for intra-hippocampal drug injection. It seems not to be precise enough for injecting a volume of 0.5 µl.

3- Minor: it is not acceptable to use two different post hoc (Tukey and Dunnet) for the same statistical analysis (ANOVA) in different experimental settings.

4- Major: in the settings one and two of the experimentations, the pain intensity was measured as a function of time and treatment. It is expected that a 2-way repeated measure ANOVA was used for data analysis in this mode, followed by an appropriate post hoc such as Bonferroni test. I suggest that the data should be re-analyzed and presented with providing detailed information including the F and P values for treatment, time, and interaction effects, and if significant, the specifics of post hoc. It would be better if the data for the 2nd experimental setting were presented with line graphs showing the pain intensities in the groups saline, NSAID, and NSAID plus naloxone over the time, including the baseline for each group.

5- Major: was it tested if the microinjection of the NSAIDs into the hippocampus...
affect the locomotor activity? Is there any previous evidence on this?

6- Major: the readers may be interested to know how the hippocampus takes part in the pain modification.

7- Minor: in the figure legends, it should be clearly mentioned that to which conditions or groups the asterisks represent differences.

8- Minor: there are a few grammatical errors in the manuscript that should be corrected.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

No